MEDICA

℞Only IVD CE

REF 10206-4 4 X 28 mL / 10 mL

ASPARTATE AMINOTRANSFERASE (AST)

Each wedge contains a usable volume of 28 mL of R1 reagent; the 10 mL bottle contains 10 mL of R2 reagent.

INTENDED USE

The EasyRA AST reagent is intended for the quantitative determination of the enzyme aspartate aminotranferase in human serum and plasma (with lithium heparin as anticoagulant), using the MEDICA EasyRA® clinical chemistry analyzer. Aspartate aminotransferase measurements are used in the diagnosis and treatment of certain types of liver and heart diseases.
For *in vitro* diagnostic use. For professional use only.

SUMMARY AND EXPLANATION

Aspartate aminotransferase is an enzyme found mainly in liver, heart, red blood cells and muscle tissue.[1] Damage to the cells of these tissues results in an increase in AST in serum that is proportional to the extent of the damage. The AST level is also markedly elevated in patients with viral hepatitis and cirrhosis and in cases of myocardial infarction.[2, 3]

PRINCIPLE OF THE PROCEDURE

The assay is based on a series of reactions described below. First, the AST enzyme catalyzes the transfer of the Aspartate amino group to α-Ketoglutarate (α -KG) with the formation of L-glutamate and oxaloacetate (OAA).

$$\text{L-Aspartate} + \alpha\text{-KG} \xrightarrow{\text{AST}} \text{OAA} + \text{L-Glutamate}$$

The OAA is then reduced to L-malate by reacting with NADH in a reaction catalyzed by malate dehydrogenase (MDH).

$$\text{OAA} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^+$$

In this second reaction the amount of NADH that is oxidized to NAD results in a decrease in absorbance at 340 nm. This decrease is followed spectrophotometrically and is directly proportional to the activity of AST in serum. Lactate dehydrogenase (LDH) is included in the reagent to rapidly reduce any pyruvate present in serum to minimize interference with the assay. The initial method was developed by Karmen[4,5] and was optimized by Bergmeyer, et al.[6] The EasyRA AST assay methodology is based on the recommended method of the IFCC.[7]

REAGENTS

AST Buffer Reagent (R1):

Tris buffer, pH 7.65 (30ºC)	110 mmol/L
L-Aspartate	320 mmol/L
LDH (microorganism)	≥1200 U/L
MDH (porcine muscle)	≥800 U/L

AST Substrate Reagent (R2):

α-Ketoglutarate	85 mmol/L
NADH (Disodium salt)	1 mmol/L

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A3).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The R1 and R2 reagents must be combined in the wedge before use. Unopened reagents are stable until the expiration date listed on the label if stored at 2 – 8°C. The working reagent is stable on-board the EasyRA clinical chemistry analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values. Protect from light.

SPECIMEN COLLECTION AND STORAGE / STABILITY
Lithium heparin coated tubes may be used for plasma collection. Clear non-hemolyzed samples should be used. AST is stable in serum or plasma for up to 7 days at 2 – 8°C.[8, 9]

Limitations
Hemolysis must be avoided, as the concentration of AST in red blood cells is approximately 10 times that of the serum.[10] Hemolyzed samples with $\geq$ 250 mg/dL hemoglobin may elevate AST values by 6 U/L or more.

PROCEDURE

Materials Provided
Medica AST Reagent Wedge/R2 Bottle, REF 10206-4

Additional Materials Required
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC® Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The R1 and R2 reagents must be combined in the wedge before use. The R1 reagent is in the wedge. Add the entire contents of the small bottle containing the R2 reagent to the wedge and mix well by inversion before use. There will be a total of 38 mL of usable working reagent after mixing. Remove the cap from the working reagent and place the reagent in the EasyRA clinical chemistry analyzer reagent tray located in the reagent area. The on-board stability (44 days maximum) of the working reagent is programmed on the RFID chip on the reagent wedge.

Note: Check inside the neck of the wedge for foam after removing the cap and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test.

Calibration
Not applicable.

Quality Control
It is recommended that two levels of human serum-based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the EasyRA clinical chemistry analyzer calculates the AST concentration from the change in absorbance per minute, sample volume, total reaction volume, pathlength (cm) of 0.6 and molar absorptivity of 6.22.

$$\text{AST (U/L)} = (\Delta A/\text{Min}) \times \frac{(\text{Total Volume}(\mu l) \times 1000)}{(\text{Molar absorptivity} \times \text{Pathlength(cm)} \times \text{Sample Volume}(\mu l))}$$

The U/L of AST activity is the amount of enzyme which oxidizes one μmol/L of NADH per minute.

EXPECTED VALUES[11]
The reference range for AST in serum is as follows:

Normal: 8 - 40 U/L (37°C)

These values are guidelines. It is recommended that each laboratory establish its own range of expected values since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g., if sample is above assay range)
Only non-hemolyzed samples should be used. Avoid using visibly turbid samples.

If the Absorbance change per Minute (ΔA/Min) is greater than 0.0643, which corresponds to 400 U/L, results will be flagged with “SD” (substrate depletion) by the analyzer. Absorbance changes per minute above this rate are above the linear range of the test. If the “Re-run” icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the AST test to 800 U/L.

PERFORMANCE CHARACTERISTS[12]

Reportable Range
The reportable range is 5.5 to 400 U/L. Extended range is 5.5 to 800 U/L when half of the sample is used (1:1 dilution).

Inaccuracy / Correlation (CLSI, EP09-A2)
The following table lists the data obtained in a comparison of the new Medica reagent for AST (y) on the EasyRA clinical chemistry analyzer to the performance of the prior Medica AST reagent (x) on the EasyRA clinical chemistry analyzer. The data shown below represents single determinations with the new Medica reagent for AST on the EasyRA clinical chemistry analyzer vs. the average of two replicate values obtained with the prior Medica AST reagent on the EasyRA clinical chemistry analyzer.

Number of samples	80	Range of Samples	10.3 to 389.5 U/L
Slope	1.0203	y Intercept	-1.183
Correlation Coefficient	0.9997	Regression Equation:	Y = 1.0203X -1.183

The following table lists the data obtained in a comparison of matched serum (x) and plasma (y) samples using the Medica reagent for AST on the EasyRA clinical chemistry analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	60	Range of Samples	10.6 to 340.4 U/L
Slope	0.9902	y Intercept	0.3133
Correlation Coefficient	0.9988	Regression Equation	Y = 0.9902*X + 0.3133

Imprecision (CLSI, EP05-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level U/L	Within Run SD U/L	Within Run CV %
234.9	1.76	0.75
102.8	0.82	0.79
37.8	1.08	2.86

Total Imprecision:

QC Level U/L	Total Imprecision SD U/L	Total Imprecision CV %
234.9	1.97	0.84
102.8	1.14	1.11
37.8	1.23	3.25

Linearity (CLSI, EP06)
Linear from 5.5 to 400 U/L, based on the linear regression Y = 0.9975*X + 0.0049

Limit of Blank (LOB):	3.1 U/L	(CLSI, EP17-A)
Limit of Detection (LOD):	4.1 U/L	(CLSI, EP17-A)

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Interfering Substances (CLSI, EP07)
Less than 10% interference was classified as “no significant interference.”
There is significant interference from hemolysis. Hemolysis must be avoided, as the concentration of AST in red blood cells is approximately 10 times that of the serum.[9] Do not use hemolyzed samples.
No significant interference was found up to 28 mg/dL of bilirubin.
No significant interference was found up to 1500 mg/dL of triglycerides (using Intralipid*).
No significant interference was found up to 30 mg/dL of ascorbic acid.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC.*

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[13, 14]

REFERENCES

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EASYRA ASSAY PARAMETERS (AST)

Wavelength (nm)	340/405 nm
Reaction type	Enzyme (0)
Reagent direction	Decrease
Reagent blank	No
Sample blank	No
Max. first interval Abs. change	0.026
Reaction time	7.2 min
Calibration interval (maximum)	N/A
Reagent on-board stability	44 days

Serum/Plasma

Sample volume (µl)	8
Diluent volume (µl)	32
Reagent volume (µl)	144
Decimal places (default)	1
Units (default values)	U/L
Dilution factor	1:1 (to extend measuring range)
Linearity	5.5 to 400 U/L